Advisory Committee on Releases to the Environment

Advice on an application for deliberate release of a GMO for research and development purposes

Applicant: The Sainsbury Laboratory

Application: To release potato lines genetically modified for resistance to potato late blight and to potato cyst nematodes and for improved tuber quality.

Ref: 19/R29/01

Date: June, 2019

Advice of the Advisory Committee on Releases to the Environment to the Secretary of State under section 124 of the Environmental Protection Act 1990

ACRE is satisfied that all appropriate measures have been taken to avoid adverse effects to human health and the environment from the proposed release. ACRE sees no reason for the release not to proceed according to the following advice.

To avoid possible adverse effects to human health and the environment, the applicant should:

1. Ensure that the GM potatoes produced as a result of this release will not be put into the human food chain or fed to livestock.

2. Ensure that any GM or non GM potato plant material remaining in the area of release at the end of the trial is inactivated.

3. Ensure that, in the two years following harvest of the GM potato tubers, the area of release is left fallow and not ploughed; but at least annual shallow tillage in the spring is used to stimulate germination of any true potato seed.

4. Treat any groundkeepers and volunteers growing from true seed in the fallow years with an application of glyphosate herbicide or hand pull potato plants prior to flowering.

5. Ensure that, during any post-trial monitoring period remaining after the fallow period, a crop is cultivated on the release site which would permit easy identification and control of groundkeepers and volunteers.

6. Control all groundkeepers and volunteers continuously until a period of four years has elapsed. Report on the number of groundkeepers and volunteers observed during this period. Appropriate herbicides should be used to control potato plants growing from true seed and from groundkeepers prior to flowering

7. Ensure a separation distance of 20 metres to non-GM potato plants growing around the trial site to minimise the possibility of cross-pollination occurring.

Comment

ACRE does not make decisions on whether to authorise the use of GM crops in field trials. It provides scientific advice to the UK regulatory authorities on any risks to the environment or to human health posed by these trials and recommends best practice for managing them, including any conditions that are required to manage a risk of harm.

Key characteristics of this release for risk assessment are:-

i) The trial will take place at two locations. The trial sites at both locations are approximately 1 000 square metres in size. TSL plans to grow a maximum of 1600 plants in each year of the trial.

ii) The GM potatoes produced as a result of this release will not be put into the human food chain or fed to livestock.

iii) GM potatoes transformed with the genetic elements described in this application have been grown in previous field trials.

TSL plans to field trial GM potato plants containing three resistance (*R*) genes. These *R* genes confer resistance to different isolates of *Phytophthora infestans*, which causes late blight in potatoes. The *R* genes are derived from wild potato relatives *Solanum venturii* and *Solanum americanum*. Some of the potato lines will contain a cystatin gene and a synthetic gene encoding a peptide that repels potato cyst nematodes. Expression of both genes has been targeted to the roots of the GM potato plants. In some cases these are stacked with *R* genes. Some plants also contain constructs that interfere with the expression of genes involved in the production of polyphenol oxidase (associated with browning of bruised tissue), the production of vacuolar acid invertase (associated with cold-induced potato sweetening) and the production of asparagine (which, in combination with reducing sugars leads to the formation of acrylamide). TSL has taken measures to limit the likelihood that vector backbone DNA is present in the GM plants. However, it has not ruled out the possibility that vector backbone DNA could be present and as such, it has included this in its risk assessment. There are two selectable marker genes on the backbones of the transformation vectors. These encode neomycin phosphotransferase II (NPTII) and isopentenyl transferase (IPT). There is also an acetolactate synthase/ chlorsulfuron resistance gene present on the transfer DNA (T-DNA) region of the transformation vectors. This facilitates the selection of potato plants containing the T-DNA, which contains the genes of interest.

ACRE has assessed GM potato plants containing these genes previously and trials involving these plants have been authorised by Defra. ACRE's view is that the risks associated with this trial are extremely low.

A number of the public representations expressed concern that TSL had not included information on the number of copies of genetic elements inserted in the GM potato plants. This was not linked to a specific risk of harm. The GMO legislation requires data to be provided on a case by case basis depending on what is required to inform the risk assessment. ACRE concluded that TSL had provided a clear description of the methods used to produce the GM potatoes and it had described the genetic elements involved in sufficient detail to perform a risk assessment. The public representations also registered concern that the number of genes inserted into the GM lines increases the potential for unexpected interactions that could result in harm. ACRE considered the potential for the different gene products or traits to interact and could not identify a plausible scientific hypothesis that would lead to harm. In addition, ACRE considered which characteristics of potato plants would need to change in order for this trial to present an increased level of environmental harm. It discussed the variation in the levels of native toxins produced by commercial potato varieties. These provide a natural defence in the field but would need to be characterised before any GM potato could be authorised for food or use as animal feed. ACRE also noted that the trial site will be monitored at least every week during the growing season for unexpected effects. Any effect that could be conceived as harmful would result in the trial being destroyed.

Late blight resistance

These *R* genes are members of the nucleotide binding site-leucine rich repeat (NB-LRR) family. This type of gene is already abundant in potato and other plant genomes. Many non-GM potato varieties cultivated in Europe already contain genes in this class. These were derived from another potato wild relative, *Solanum demissum*. Introduction of these additional *R* genes to the GM potatoes is intended to increase the range of late blight pathotypes to which potato is resistant. There is no evidence of *R* genes of the NB-LRR family conferring toxic or allergenic properties. The corresponding promoter and terminator regions from the potato wild relatives have been transferred along with the *R* genes.

Potato cyst nematode (PCN) resistance

Some of the GM potato lines used in this trial will produce a PCN-repellent peptide that disrupts the chemoreception that PCN require to locate host plants and a cystatin that limits the growth of PCN by inhibiting digestive enzymes (cysteine proteinases). GM potatoes containing these genes have been trialled in England previously without unintended adverse effects. The results of previous trials and laboratory experiments are presented in peer-reviewed papers by Lilley et al (2004)[1], Kiezenbrink and Atkinson (2004)[2] and

[1] Lilley C.J, Urwin P.E, Johnston K.A, Atkinson H.J. (2004) Preferential expression of a plant cystatin at nematode feeding sites confers resistance to *Meloidogyne incognita* and *Globodera pallida*. Plant Biotech J. 2: 3–12.

[2] Atkinson HJ, Keizenbrink DT (2004) Determining risks to soil organisms associated with a genetically modified (GM) crop expressing a biopesticide in its roots. Available at: http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&Completed=0&ProjectID=11999.

Green et al (2012)[3]. These show that the peptide and the cystatin offer potential for controlling PCN without impacting on the non-target nematode soil community.

Gene-silencing (RNA interference)

The transformation vectors contain fragments of three native potato genes that when expressed, are designed to silence the polyphenol oxidase gene (*Ppo*), the asparagine synthetase-1 gene (*Ast1*) and the vacuolar acid invertase gene (*Vlnv*) in the potato tubers. The aim is to produce potato tubers that do not brown on bruising and which are less likely to blacken or produce acrylamide upon cooking. A number of public representations were concerned that there is no specific guidance from the European Food Safety Authority (EFSA) on using gene silencing in GM crops, which would apply if a company applied to cultivate these GM potatoes on a commercial-scale or to market them as human food or animal feed products.

ACRE noted that in 2015, the US and Canadian Governments completed their assessment of and deregulated so-called 'Arctic apples'. *Ppo* gene expression is silenced in these apples so that they do not brown when sliced. The US's Food and Drug Administration completed its assessment on whether Arctic apples are as safe and nutritious as their conventional counterparts at the same time as it completed its assessment of 'Innate' potatoes. These potatoes contain the gene-silencing molecules present in the GM potatoes involved in the trial proposed by TSL. These have not been authorised for food and feed use in the EU but other GM products in which gene silencing has been used to produce beneficial traits have. These include MON 87705 and 305423 soybean. These GMOs produce seeds with lower levels of saturated and polyunsaturated fatty acids and higher levels of a healthier monounsaturated fatty acids. The regulatory assessment of these GMOs for food and feed use in the EU includes an environmental safety assessment for low levels of environmental presence. Public representations highlighted that these are not authorised for commercial cultivation in the EU.

Gene silencing does not result in novel proteins. A concern raised in public representations was that genes other than the target genes would be affected and that this would alter the GM potatoes in a way that will cause environmental harm. These GM potatoes will not enter the food/ feed chain; they would need to be assessed and authorised for this use. Therefore, ACRE considered what characteristics of these GM potatoes could be altered to result in environmental harm when grow on a small-scale under the conditions of this trial. ACRE noted that potatoes produce glycoalkaloids, which are natural toxins that provide protection from herbivores and fungi. The levels can vary in commercial varieties and would be checked as part of the GM food/ feed safety assessment. ACRE noted that TSL will monitor the trial site (at least) weekly during the trial and will have a legal obligation to inform regulators of unintended effects. ACRE did not identify any plausible risks of harm that TSL should monitor for specifically.

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[3] Green.J. Wang D, Lilley C.J, Urwin P.E, Atkinson H.J. (2012) Transgenic Potatoes for Potato Cyst Nematode Control Can Replace Pesticide Use without Impact on Soil Quality. PLoS ONE. 7(2): 1- 9. e30973

The public representations also raised concern that species feeding on these plants might be harmed if the gene silencing molecules they ingested inhibited the expression of native genes. Organisms feeding on fresh plant matter inevitably consume considerable quantities of small active RNAs in their diets. Whether these molecules then enter cells and regulate native gene activity is strongly debated. Studies reporting the uptake and function of small RNAs are controversial in the scientific community. This includes the high profile report of a plant-derived micro RNA (MIR168a) affecting cholesterol homeostasis in mammals[4]. A recent review of all data in this field is very critical of the concept[5]. In particular, there is strong evidence of contamination in sequencing datasets[6] a problem acknowledged by the originators of the concept in their most recent review[7].

In the case of this field study, material will not enter the animal feed or human food chains. If the area of the trial was considerably larger (e.g. if plants producing these small RNAs were grown on a commercial-scale) a more detailed assessment of the impact on species exposed to these molecules is likely to be required, noting that pest species will have the highest levels of exposure.

Antibiotic resistance marker genes

TSL's use of the *nptII* antibiotic resistance marker gene was raised in most of the public representations on this application. TSL is using *nptII* gene expression to select for the presence of two of its plant transformation vectors in bacterial cells. NPTII confers resistance to the antibiotics neomycin and kanamycin, which are included in the medium on which the bacteria are cultured. By including the *nptII* gene in the backbone of these vectors and selecting for GM plants that do not contain vector backbone, TSL intends to use GM potato plants in the trial that do not contain the *nptII* gene. This is likely to be helpful if any of these GM potato lines are developed for commercial cultivation in the future. ACRE has considered the consequences of the *nptII* gene being present in GM plants used in small-scale trials on a number of occasions previously, including after a statement by the European Medicines Agency (EMA) on the importance of preserving the therapeutic relevance of the antibiotics kanamycin and neomycin. ACRE remains of the opinion that the therapeutic effect of antibiotics that are substrates for NPTII will not be compromised by the presence of the *nptII* gene in GM plants. ACRE's advice on this issue is that (i) the likelihood of transfer of a functional gene from plant material to bacteria is extremely low and that (ii) bacteria with resistance to these antibiotics are widespread in the environment.

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[4] Zhang L, Hou D, Chen X, Li D, Zhu L, Zhang Y ,Li J, Bian Z, Liang X, Cai X, Yin Y, Wang C, Zhang T, Zhu D, Zhang D, Xu J, Chen Q, Ba Y, Liu J, Wang Q, Chen J, Wang J, Wang M, Zhang Q, Zhang J, Zen K, Zhang C-Y. (2012). Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross- kingdom regulation by microRNA. Cell Research. 22(1):107–26.

[5] Chan S.Y.and Snow J.W. (2017). Formidable challenges to the notion of biologically important roles for dietary small RNAs in ingesting mammals. Genes and Nutrition. 7: 12-13

[6] Tosar J.P, Rovira C, Naya H, Cayota A. (2014). Mining of public sequencing databases supports a non-dietary origin for putative foreign miRNAs: underestimated effects of contamination in NGS. RNA. 20(6):754–7.

[7] Zhang L, Chen T, Y Yin, C-Y Zhang and Y-L Zhang (2019). Dietary microRNA—A Novel Functional Component of Food. Advanced Nutrition 00:1–11,

TSL have used the tomato acetolactate synthase (*als*) gene as a plant selectable-marker to distinguish the GM potatoes from untransformed potatoes in tissue culture. This gene encodes a variant of the ALS enzyme that confers resistance to sulfonylurea and imidazolinone herbicides. There are a number of crops developed through traditional breeding methods that have resistance to sulfonylurea and imidazolinone herbicides as a result of mutations in the ALS gene including oilseed rape and wheat varieties. These plants will be sensitive to other herbicides such as glyphosate or glufosinate, which could, if necessary, be used to eliminate them in the field trial. Sulfonylurea and imidazolinone herbicides should not be used at the trial site. The potential for horizontal gene transfer (HGT) from plants to soil bacteria has been discussed above with respect to the *nptII* gene.

TSL is also using a gene encoding isopentenyl transferase (*ipt*) to identify GM potato plants containing vector backbone. The *ipt* gene is present in two of the plant transformation vectors, just outside of the left borders. IPT is a cytokinin and GM plants producing it will have a characteristic shooting phenotype, which is easily detected and the plants discarded.

Pollen-mediated gene flow to other plants

Cultivated potatoes are a low-risk crop for pollen-mediated gene flow because they are highly self-compatible and cannot cross with other wild or ornamental species in the UK to produce viable offspring. Successful transfer of pollen from the GM potatoes to a non-GM potato growing in a commercial crop relies on pollen being transported by the wind or by insects. ACRE recognises that rare long-distance cross-pollination events are possible, especially where pollen beetles are common in the area of the trial site. However, cross-pollination frequencies reduce dramatically over distance and pollen competition from within a non-GM potato crop reduces the likelihood of successful hybridisation further. Even if GM pollen successfully hybridised and resulted in GM seed, the chance of such seed successfully germinating and surviving until harvest as a tuber in a non-GM potato crop is low because potatoes are usually grown in rotations and the volunteers resulting from true seed are very vulnerable to herbicide applications and crop competition.

Therefore, ACRE considers that the proposed separation distance of 20 metres to non-GM potato plants growing around the trial site is appropriate. Public representations noted that TSL is not proposing to use guard rows of non-GM potatoes to reduce edge effects, which can compromise the trial. This was a feature of previous trials. ACRE does not consider that a non-GM border row is necessary to further minimise the likelihood of pollen from the GM plants pollinating potato plants in the vicinity of the trial for the reasons discussed in the previous paragraph.

ACRE considers that the measures proposed by TSL to inactivate material from the trial site are appropriate. These should be applied to viable non-GM as well as GM material on the trial site. ACRE also notes that the trial will be overseen by the GM Inspectorate and that it is appropriate given the experimental nature of the programme of work for details of the plot design to be provided just prior to the time of the release.

Monitoring of previous releases of potatoes has revealed that groundkeepers may persist for several years after the initial release. These derive from tubers or fragments of tubers left in the soil after harvesting. Where there is sufficient space between plots, the use of a mechanical lifter is likely to be more efficient in removing tubers. Hand-pulling will be preferable if tubers from surrounding plants have the potential to ricochet off the machinery because of their proximity. ACRE considers that potato plants arising from true seed are unlikely in agriculturally managed situations. However, as a precautionary measure, ACRE advises that the trial site be managed so that any potential for true seed to persist and germinate into GM plants in future years is also minimised. ACRE advises that the trial site should be harvested, and potato tops removed from the field, as soon as practical after results have been obtained in order to minimise maturation and any potential shedding of true seed. In addition ACRE advises that the ground on which potatoes have been released should remain fallow for two years following the release and not ploughed. This would allow true potato seed and tubers to remain near the soil surface and produce volunteers. Light tillage should be carried out annually in the spring to stimulate germination of true potato seed but no other form of cultivation should be used on the release area. After two years, crops that facilitate the removal of potato groundkeepers and volunteers should be grown throughout the remaining post-trial monitoring period. ACRE considers that TSL should monitor the trial plots for potato groundkeepers and volunteers for at least four years after they are harvested. At the end of four years, the results should be examined to determine whether monitoring can stop.

Items arising from public representations

ACRE has addressed many of the comments relating to the potential for this particular trial to cause harm to human health and the environment in previous sections of this advice. This does not include a food safety assessment as any consent that is issued for this trial will not permit material from the trial to enter the food or animal feed chains. ACRE has not addressed issues that are not safety concerns such as whether the GM potatoes will be of benefit to consumers and whether there are better options for addressing the agronomic and health challenges these GM potatoes were developed to tackle.

Some of the representations raise concerns about the commercial cultivation of these GM potatoes, for example whether farmers might use sulfonylurea and imidazolinone herbicides. If these GM potatoes were notified for commercial cultivation in the future, such concerns would be considered. ACRE is required to advise on the risks posed by this particular trial and agrees that these herbicides should not be used on the trial site.

ACRE is grateful to those who have submitted comments and has used them in considering the safety of this trial.